

1 What is claimed is:

2 1. A method of using Raman imaging microscopy for evaluating drug action  
3 within living cells comprising the steps of:

4 first measuring a Raman spectrum of said drug to determine a pretreatment  
5 fingerprint of said drug;

6 second measuring a Raman spectrum of said living cells in order to  
7 determine a pretreatment fingerprint of said living cells;

8 treating a culture of said living cells with said drug to obtain treated living  
9 cells;

10 next measuring a Raman spectrum of said treated living cells to obtain post-  
11 treatment images of said living cells;

12 processing said post-treatment images and pretreatment fingerprints to obtain  
13 processed post-treatment images and processed pretreatment fingerprints; and

14 dividing said post-treatment images by said processed pretreatment  
15 fingerprints to obtain a ratio of images which indicate the changes of said living cells  
16 after said treating whereby said changes are used to determine said drug action.

17 2. The method of using Raman imaging microscopy for evaluating drug action  
18 within living cells of Claim 1 wherein said ratio of images is obtained for various  
19 times to determine different depths within said living cells.

20 3. The method of using Raman imaging microscopy for evaluating drug action  
21 within living cells of Claim 2 further comprising the step of stacking said ratio of

- 1 images to obtain a three dimensional Raman image.
- 2 4. The method of using Raman imaging microscopy for evaluating drug action  
3 within living cells of Claim 3 wherein said processing step comprises determining  
4 the Raman scattering coefficient of an imaging area from a recorded image for said  
5 post-treatment images and said pretreatment fingerprints.
- 6 5. The method of using Raman imaging microscopy for evaluating drug action  
7 within living cells of Claim 4 wherein said processing step further comprises  
8 determining said Raman scattering coefficient by reducing any noise from said  
9 recorded image.
- 10 6. The method of using Raman imaging microscopy for evaluating drug action  
11 within living cells of Claim 5 wherein said processing step further comprises  
12 determining said Raman scattering coefficient by compensating for any point spread  
13 function.
- 14 7. The method of using Raman imaging microscopy for evaluating drug action  
15 within living cells of Claim 6 wherein said processing step further comprises  
16 determining said Raman scattering coefficient by eliminating any non-uniform  
17 illumination and subtracting any fluorescent background from said recorded image.
- 18 8. The method of using Raman imaging microscopy for evaluating drug action  
19 within living cells of Claim 2 further comprising the step of plating said living cells  
20 on a dish coated with Raman inactive material to prevent Raman signals coming  
21 from said dish during said measuring steps.

- 1 9. The method of using Raman imaging microscopy for evaluating drug action  
2 within living cells of Claim 8 wherein said treating step occurs for at least one hour.
- 3 10. The method of using Raman imaging microscopy for evaluating drug action  
4 within living cells of Claim 9 wherein said measuring steps utilize a system having a  
5 Raman microscope with a 30 mw diode laser at 780 nm as the excitation source.
- 6 11. The method of using Raman imaging microscopy for evaluating drug action  
7 within living cells of Claim 10 wherein said system is stabilized on a vibration  
8 controlled table.
- 9 12. The method of using Raman imaging microscopy for evaluating drug action  
10 within living cells of Claim 11 wherein said living cells are obtained with a water  
11 immersion, high infrared transmission objective.
- 12 13. The method of using Raman imaging microscopy for evaluating drug action  
13 within living cells of Claim 12 wherein said transmission objective has a  
14 transmission coefficient of the lens at 780 nm excitation wavelength of 71%.
- 15 14. The method of using Raman imaging microscopy for evaluating drug action  
16 within living cells of Claim 13 wherein said lens has a numerical aperture of 0.90.